Documentation of cunner (Tautogolabrus adspersus) cleaning behaviour in tanks with Atlantic salmon (Salmo salar) smolts infested with sea lice (Lepeophtheirus salmonis)

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2016

Canadian Technical Report of
Fisheries and Aquatic Sciences 3168

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Canadian Technical Report of Fisheries and Aquatic Sciences

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Canadian Technical Report of
Fisheries and Aquatic Sciences 3168

2016

DOCUMENTATION OF CUNNER (*TAUTOGOLABRUS ADSPERSUS*) CLEANING BEHAVIOUR IN TANKS WITH ATLANTIC SALMON (*SALMO SALAR*) SMOLTS INFESTED WITH SEA LICE (*LEPEOPHTHEIRUS SALMONIS*)

by

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Cat. No. Fs97-6/3168E-PDF ISBN 978-0-660-05605-0 ISSN 1488-5379

Correct citation for this publication:

Costa, I., Hamoutene, D., Murray, H.M., Lush, L., Burt, K., Eaves, A. and P. Keng. 2016. Documentation of cunner (*Tautogolabrus adspersus*) cleaning behaviour in tanks with Atlantic salmon (*Salmo salar*) smolts infested with sea lice (*Lepeophtheirus salmonis*). Can. Tech. Rep. Fish. Aquat. Sci. 3168: iv + 11 p.

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ABSTRACT

Costa, I., Hamoutene, D., Murray, H.M., Lush, L., Burt, K., Eaves, A. and P. Keng. 2016. Documentation of cunner (*Tautogolabrus adspersus*) cleaning behaviour in tanks with Atlantic salmon (*Salmo salar*) smolts infested with sea lice (*Lepeophtheirus salmonis*). Can. Tech. Rep. Fish. Aquat. Sci. 3168: iv + 11 p.

The delousing efficiency of cunner (*Tautogolabrus adspersus*) against *Lepeophtheirus salmonis* infecting Atlantic salmon (*Salmo salar*) is documented in a tank study through the assessment of cunner behavioural responses. Lice counts are reduced by 89.3% in salmon smolts after 78 hours residence with cunners (2 tanks; cunner to salmon ratio of 1:10); as compared to the control tank without cunners that did not show a significant reduction of lice over the same time period ($p=0.275$). Video data show a significant increase of cunner activity (salmon chasing, and lice picking) with cohabitation time with salmon. This study confirms the lice cleaning efficiency of cunner through the documentation of behavioural responses in a tank study. Findings suggest that cleaning is likely an opportunistic behaviour not occurring naturally in cunner, but easily acquired through cohabitation with salmon.

RÉSUMÉ

Costa, I., Hamoutene, D., Murray, H.M., Lush, L., Burt, K., Eaves, A. and P. Keng. 2016. Documentation du comportement de nettoyage de la tanche-tautogue (*Tautogolabrus adspersus*) dans les réservoirs contenant des saumoneaux de l'Atlantique (*Salmo salar*) infestés par le pou du poisson (*Lepeophtheirus salmonis*). Can. Tech. Rep. Fish. Aquat. Sci. 3168: iv + 11 p.

L'efficacité de la tanche-tautogue (*Tautogolabrus adspersus*) contre le pou du poisson (*Lepeophtheirus salmonis*) infectant le saumon de l'Atlantique (*Salmo salar*) est documentée dans une étude de réservoirs qui évalue les réponses comportementales des tanches-tautogues. Le nombre de poux diminue de 89,3 % chez les saumoneaux après 78 heures de cohabitation avec des tanches-tautogues (deux réservoirs; proportion de tanches-tautogues par rapport au nombre de saumons de 1 pour 10), tandis que dans le réservoir témoin sans tanches-tautogues, on n'a pas constaté de diminution importante du pou du poisson au cours de la même période ($p = 0,275$). Les données vidéo montrent une augmentation importante de l'activité des tanches-tautogues (chasse des saumons et élimination des poux) durant leur cohabitation avec les saumons. En documentant les réponses comportementales dans une étude de réservoirs, cette étude confirme l'efficacité de la tanche-tautogue pour l'élimination du pou du poisson. Les constatations laissent supposer que le nettoyage est probablement un comportement opportuniste qui n'est pas naturel chez la tanche-tautogue, mais qu'elle acquiert facilement lorsqu'elle cohabite avec le saumon.

INTRODUCTION

In many salmon farming countries such as Canada, Norway, Scotland, and Ireland, prolonged use of chemical therapeutants (eg. SLICE) to control sea lice (*Lepeophtheirus salmonis*) infestation has led to the emergence of resistance in local lice populations (Denholm et al. 2002). The utilization of cleaner fish as an alternative to chemical treatments for control of sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) has been investigated since the late 1980's and early 1990's. Much of the initial work was carried out by Bjordal (1988; 1990; 1991; 1992) and involved primarily wrasse species common in Northern Europe (i.e Corkwing wrasse (*Symphodus melops*) , gold sinny wrasse (*Ctenolabrus rupestris*), juvenile ballan wrasse (*Labrus bergylta)*, rock cook wrasse (*Centrolabrus exoletus*), and the female cuckoo wrasse (*Labrus mixtus*)). Initial results showed that, while cleaning efficiency varied between species, wrasse were observed to be quite effective at cleaning lice in both controlled tank and commercial cage level experiments (Costello 1993; Treasurer 1994; Tully et al. 1996). In North America, evaluation of the potential of cleaner fish to control sea lice is in its infancy compared to what has taken place in Europe. MacKinnon (1995) described the first study evaluating the potential of cunner *Tautogolabrus adspersus* as lice cleaners; however, the author did not document in detail the cunners behaviour in tanks. The cunner is a labrid inhabiting North Atlantic coastal waters from Chesapeake Bay to Northern Newfoundland (NL) (Leim and Scott 1966) and is particularly common around insular Newfoundland. They mature at 8-11 cm in length, grow to a maximum of 43 cm, and may live up to 10 years (MacKinnon 1995). The objectives of the present study are to describe the lice cleaning efficiency of cunners in a tank study through the assessment of behavioural responses.

MATERIAL AND METHODS

SEA LICE INFESTATION

One hundred and fifty integrated transponder (PIT) tagged farmed Atlantic salmon smolts (average total length 210 mm; average mass 148 g) were acclimatized for 4 weeks into three 1364 L tanks (50 salmon tank^{-1}) supplied with flow-through ambient seawater (average of 11.4°C) under ambient photoperiod (end of October). Adult sea lice (*Lepeophtheirus salmonis*), collected the day prior from salmon held in sea cages of NL south coast were introduced to all 3 salmon tanks to initiate lice infestation. After lowering the water level to 50% capacity, 300 adult sea lice (100 lice tank^{-1}) were introduced to the tanks similar to previous studies (i.e Mackinnon 1995). Tanks were filled back to capacity after one hour. Five days post-infestation, individual fish were netted,

anesthetized with buffered TMS (MS-222), identified by PIT tag, and the total number of lice present on each salmon counted. At twelve days post-infestation 5 cunners (*Tautogolabrus adspersus*) were added to 2 of the tanks (cunner to salmon ratio 1:10); the 3rd tank did not receive cunners and was used as control. The cunners used in this experiment were sourced from Placentia Bay, NL, and resident at the North Atlantic Fisheries Centre for 2 years (average total length: 14.7 cm). Seventy eight hours (3 days and a morning) post-cunner introduction, salmon were individually removed as previously described, and lice counts were recorded again.

CUNNER BEHAVIOURAL ASSESSMENT

Following the introduction of cunners to all tanks videography began immediately using Go-Pro Hero 3 cameras in an underwater casing mounted at the surface of the tanks. Video recording was continuous during daylight periods (average 12 hr/ day) over a 78 hour trial period (3 days and a morning). No feed was presented to the fish during the trial. Four randomly selected video segments of 5 minutes were reviewed for each day with two segments in the morning and two during the afternoon using Observer XT software (Noldus Information Technology, Wageningen, Netherlands). Specific cunner behaviours were identified and quantified (i.e. occurrence) as follows: 1) occurrence of "flash" swimming (rapid sudden movements) throughout the day, 2) occurrence of cunner chasing salmon, and 3) lice picking attempts (n=4 counts per day for 3 days, one count for every 5 min segment viewed, 2 counts for the last morning). In addition, after every minute of elapsed video, videos were halted and numbers of fish swimming and resting in the frame counted (20 counts per day, 10 for the last morning).

STATISTICAL ANALYSES

Due to tank restrictions and timely access to fish and sea lice, our trials (with or without lice) consisted of one control and two experimental (with cunners) tanks. We did not compare tanks due to the lack of tank replication. Our statistical approach is based solely on the assessment of change in lice counts (salmon) or fish behaviour (cunners) with time within every tank (results are presented per tank). Two-tailed paired t-tests (repeated measures with time, i.e. same fish), or a Wilcoxon Signed Rank test (if the data normality assumption failed), was used to detect significant differences between initial and final lice counts in the salmon smolts. In order to investigate the effect of cohabitation time on cunner behaviour, a series of Pearson Product correlations with time was completed for

each tank for occurrence of flash swimming, salmon chasing, and lice picking. Time is expressed as incremental observations from start of filming up to end of the morning of the 4th day. There are a total of 14 observations (1 to 14) for the occurrence of behaviours counted over 5 min video segments (4 for every day, 2 for the last day) and 70 (1 to 70) observations for percentages of swimming cunners (one for every minute of filming, 10 minutes only for the last day). Two way ANOVAS with day (1 to 4) and time of day (AM or PM) as the two factors were used to compare percentages of swimming cunners. All statistical tests were performed using Sigma Stat 12.5 (Systat Software, Inc) with α=0.05.

RESULTS

LICE COUNTS

Paired t- test (fish are identified individually) results show a significant reduction in lice counts on salmon smolts after 78 hours residence with cunners, P=0.008 for experimental tank 1 (Exp.1), and P<0.0001 for experimental tank 2 (Table 1). Lice counts were reduced by 82.6% and 97.5% in the experimental tank 1 and 2 respectively. There is also a non-significant loss of adult lice in the tank without cunners of 43.3%.

Table 1- Comparison of lice counts (means ± SD or SE? and totals) in the control (without cunners) and experimental tanks (with cunners)

Treatment/tank	Initial lice count per fish	Lice count per fish after 78h	P (paired t-tests)	Initial total lice count	Total lice count after 78h
No Cunners (N=50)	0.53±1.30	0.30±0.64	0.275	27	15
With Cunners Exp1 (N=44**)	0.46±0.92	0.08±0.28*	0.008	22	4
With Cunners Exp2 (N=43**)	0.79±1.12	0.02±0.15*	P<0.0001	37	1

* denotes a significant difference. Exp.: experimental tank (1 or 2)

** The remaining 6 or 7 smolts did not have lice on them.

CUNNERS BEHAVIOUR

Continuous filming during daylight hours provided documentation of cunner lice picking behaviour in the presence of salmon (Figure 1). Frames taken every minute allowed us to determine the number of cunners swimming per minute. At any given time during the day (AM or PM) 20.0 to 56.0% of the cunners observed in the frame were swimming. Comparisons of AM and PM values of percentages

per day were completed using two-way ANOVAS with day and time of day as factors (results not shown) and showed no differences in proportions due to time of day.

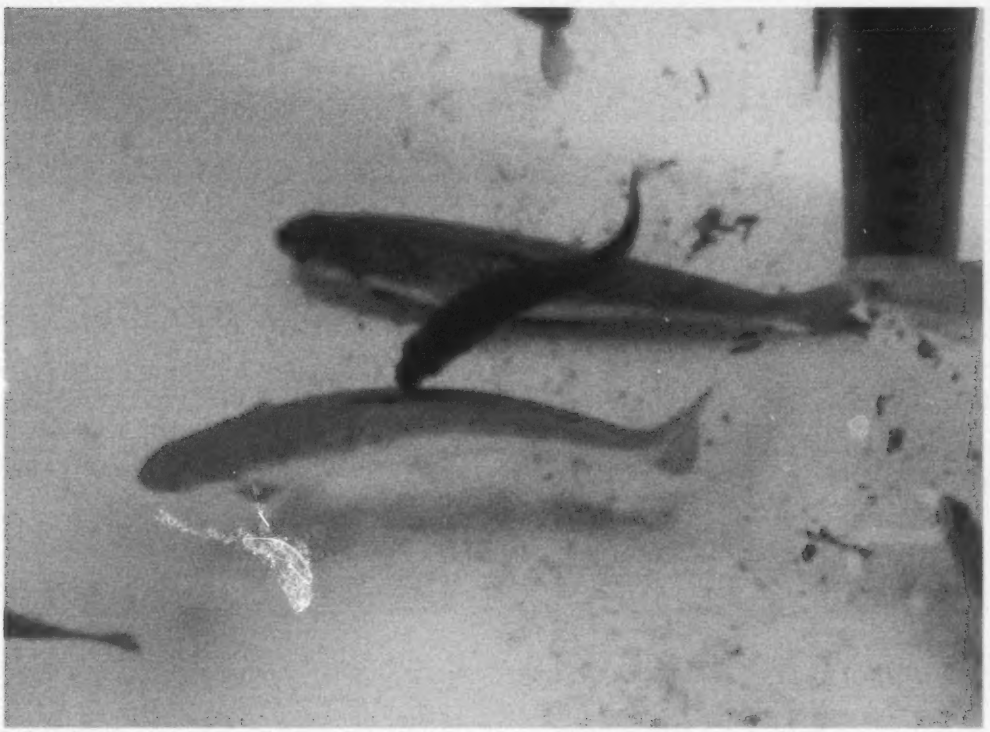

Figure 1- Still of a cunner picking lice from a salmon.

Table 2- Average occurrence of behaviours (flash swimming, lice picking, and salmon chasing) in experimental tanks (first 3 days). AM: average of the two 5 min video segments recorded in the morning; PM: average of the two 5 min video segments recorded in the afternoon.

	Videos	Flash swimming	Lice picking	Salmon chasing	Average duration (s) of chasing per cunner (± SD)	Average % cunners swimming per minute (± SD)
Experimental tank 1	AM Day1	0.0	0.0	0.5	6.2 ± 3.1	24.0 ± 22.7
	PM Day1	1.0	0.0	0.5		30.0 ± 25.4
Experimental tank 2	AM Day1	1.0	1.5	3.5	3.0 ± 2.1	44.0. ± 24.6
	PM Day1	0.5	0.0	2.5		42.0 ± 22.0
Experimental tank 1	AM Day2	0.0	0.0	1.5		20.0 ± 21.1
	PM Day 2	0.0	1.5	5.0	3.3 ± 1.2	38.0 ± 23.9
Experimental tank 2	AM Day 2	0.0	3.5	10.5		56.0 ± 24.6
	PM Day2	1.0	2.0	4.0	14.6 ± 30.3	44.0 ± 26.3
Experimental tank 1	AM Day3	0.5	0.0	2.5		46.0 ± 19.0
	PM Day3	0.5	1.0	4.0	2.1 ± 1.4	46.0 ± 16.5
Experimental tank 2	AM Day3	0.0	1.5	5.0		42.0 ± 19.9
	PM Day3	1.0	2.0	7.0	3.0 ± 2.1	42.0 ± 23.9
Experimental tank 1	AM Day4	0.0	5.0	4.5	12.1± 9.9	38.0 ± 19.9

	AM Day 4	0.5	4.0	5.0	5.3 ± 2.7	42.0 ± 22.9
Experimental tank 2						

Correlations with cohabitation time were explored for occurrences of specific behaviours and percentages of active cunners. Overall, our observations show an increase in picking and chasing activity of the cunners with cohabitation time with salmon (Table 3, Figure 2). We can note some fish behavioural differences between tanks: cunner lice picking did not start on the first day for experimental tank 1 and had three occurrences for the 4 video segments in tank 2. Similarly, a positive correlation of percentages of swimming cunners with cohabitation time was found significant for experimental tank 1 only (Table 3).

Table 3- Correlations between cunner behaviours and cohabitation time 4)

Tank	Salmon chasing	Lice picking	Flash swimming	Percentage of swimming cunners
Exp1	R=0.834 P<0.001*	R=0.837 P<0.001*	R=-0.143 P=0.626	R=0.445 P<0.001*
Exp2	R=0.534 P=0.048*	R=0.493 P=0.073	R=-0.284 P=0.325	R=-0.098 P=0.421

* denotes a significant correlation with time. Exp: experimental tank (1 or 2). R: correlation coefficient. P=probability.

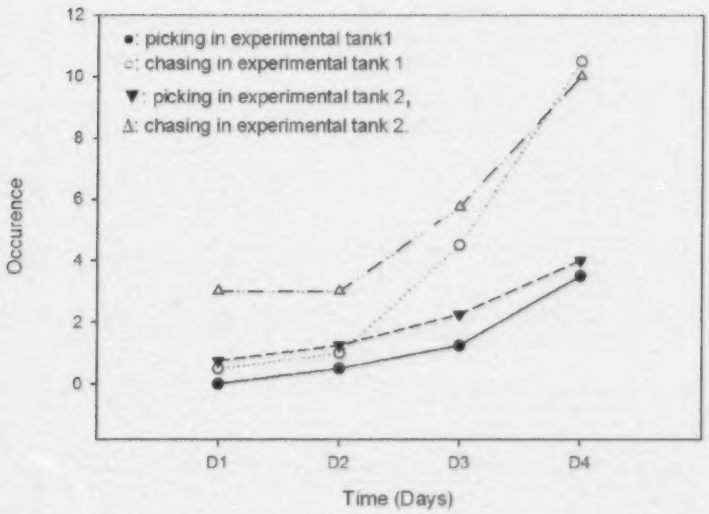

Figure 2- Occurrences (average number of times behavior occurs per day) of cunner behaviours with cohabitation time in days (D). Every point represents the mean of four observations for that day.

DISCUSSION

The objective of the present study was to document the behaviour of NL sourced cunners (*Tautogolabrus adspersus*) when picking sea lice (*Lepeophtheirus salmonis*) from farmed Atlantic salmon smolts held in an enclosed tank system. Our data demonstrate that under the conditions of our experiment NL cunners do effectively remove adult *Lepeophtheirus salmonis* from Atlantic salmon smolts (Figure 1). In a similar attempt to demonstrate the effectiveness of New Brunswick source cunner to clean *Caligus elongatus* from infected Atlantic salmon smolts Mackinnon (1995) exposed a single infected salmon to a single cunner in 136 l tanks over a course of 20 trials. These trials showed that lice numbers decreased significantly in the presence of cunners as compared to when two salmon were held concurrently. In our study, lice counts were reduced by 81.8% and 97.3% in the experimental tank 1 and 2 respectively after individual fish were surveyed before and after cunner introduction. However observed a substantial though non- significant (P=0.275) decrease in adult lice counts in the control tank. We cannot exclude losses due to handling, use of anesthetic, host rejection and/or lice mortalities. Glover et al. (2004) noted a minimal (3.0-3.6%) loss of lice in the anesthetic bath used prior to counts. Lice rejections are believed to be due in part to cellular/skin responses and/or possible physical

removal by fish rubbing against tanks (Johnson and Albright 1992). A caveat to our findings resides in the lack of replication of the control tank and the fact that only two experimental tanks were used. Other authors have noted large differences in infestation when setting up tank trials (e.g. Tucker 1998; Tucker et al. 2002). Fast et al. (2006) suggest that a minimum of 4 replicate tanks be used in studies especially when evaluating stress response of fish.

Our observations show a significant positive correlation between cunner cleaning behaviour and cohabitation time with salmon (Table 3, Figure 2). This "learning curve" shows that cleaning is likely an opportunistic behaviour not occurring naturally in the wild. Cleaning behaviour by captive fishes may differ according to species; in a comparison of the cleaning behavior of Goldsinny and Rockcook wrasses in a tank experiment, Tully et al. (1996) found that the Rockcook started cleaning immediately and was more active and enthusiastic in its initiation of cleaning whereas the Goldsinny was timid and less interactive. However, we can note some fish behavioural differences between tanks: cunner lice picking did not start on the first day for experimental tank 1 and had three occurrences for the 4 video segments in tank 2. This confirms previous observations by MacKinnon (1994) that cleaning behaviour in cunners may be a feature also determined by individual experiences rather than a typical species-wide behaviour. Furthermore, the performance of cleaner fish in captivity is likely influenced by their familiarity with the cage/tank conditions allowing cleaning behaviours to occur faster when fish are acclimatized to the particular environment where salmon are held. Deady et al (1995) suggest that this adaptation to the tank or cages where salmon are held might be necessary for some species to initiate cleaning interactions. Donnelly (1992) reported a 2-3 week interval before wrasse, taken directly from the wild, became accustomed to the cage situation; Deady et al (1995) noted a quicker acclimatization of wrasse in salmon cages due to their maintenance in a holding cage some time beforehand. Over the course of the study no direct aggressive behavior was observed (cunner to salmon or salmon to cunner). Bjordal (1991) indicated that the Rockcook wrasse showed aggression to the point of wounding salmon. Reciprocal aggressive behaviour of salmon towards the wrasse was also observed when the cleaner fish were first introduced to the cages (Glover et al. 2004). It is worth noting that in the present study salmon did not seem to avoid interactions with cunners. This is similar to the Losos et al., (2010) study on sticklebacks, where authors conclude that there is no coercive relationship, whereby sticklebacks (or cunners in this study) take clear advantage of the salmon's inability to flee while in the experimental tanks. Only 20 to 46% of cunners exhibit swimming activity during the observation period. If we assume that this level of activity is linked to a potential picking behaviour, our results

confirm MacKinnon (1995) observations that only a proportion of the cunners show active cleaning behaviour.

To conclude, this study suggests that NL Cunners will remove adult *Lepeophtheirus salmonis* from Atlantic salmon smolts when compared to a control tank without Cunners. These encouraging results indicate more extensive trials would be worthwhile. Further work could include an evaluation of the effects of different sizes of cunner and or salmon as well as the effectiveness of the cunner in picking lice of different sizes or stages. Optimization of the ratio of cunner to salmon would also be helpful as would an evaluation of whether pre-adaptation with salmon would improve picking efficiency. Cage trials by MacKinnon (1995) with 30 cunners to 2000 Atlantic salmon have shown no reduction in lice numbers. However, these trials were conducted at a low cunner to salmon ratio of 1:66, and are likely influenced by the availability of fouling organisms on the cage nets, which can form a dietary distraction from lice picking (MacKinnon 1995). Further work is needed in the cage environment in the light of recent improvements in net cleaning while addressing number of cunners per salmon, pre-adaptation of cunners to the cage environment, and other potential cleaning distractions (salmon feed).

ACKNOWLEDGEMENTS

This study was supported by the Aquaculture Collaborative Research Development Program (ACRDP).

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